IMMUNOLOGICAL MARKERS IN ADULT PATIENTS WITH EPILEPSY

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OULUN YLIOPISTO, OULU 2005
Abstract

Increased prevalence of anticardiolipin antibodies (aCL) and antinuclear antibodies (ANA) and changes in serum immunoglobulin concentrations have been reported in patients with epilepsy. The purpose of this study was to determine the presence of aCL, ANA, anti-B2 glycoprotein I-antibodies (anti-B2-GPI), antimitochondrial antibodies (AMA), immunoglobulin A, G and M serum concentrations and the presence of IgA and IgG class antigliadin antibodies (AGAbA and AGAbG), transglutaminase antibodies (tTGAbA) and antiendomysial antibodies (EMA) in a cohort of 1386 adult patients treated for epilepsy in the Oulu University Central Hospital during the years 1996–7 and in a reference population obtained from the Population Register Centre and matched for age, gender and municipality of residence. The effects of co-morbidity, medications, age, gender and different epilepsy attributes on the occurrence of the immunological parameters studied as well as the possible interrelations of these parameters were studied.

There was no difference in the presence of aCL or ANA between the patients and the reference subjects. In patients, aCL were associated with long duration of epilepsy and poor seizure control. Low IgA serum concentrations were more common in patients with epilepsy, particularly those using phenothyin. Unspecific AMA were more common among the epilepsy patients. The prevalence of coeliac disease (CD)-related antibodies was similar in patients with epilepsy and in the reference population. AGAbA were associated with primary generalised epilepsy. No significant interrelations between the immunological markers were found. These findings suggest that patients with epilepsy do not have an increased prevalence of autoantibodies as a result of their disease. Various factors such as genetic traits and epilepsy attributes may independently affect the presence of each individual immunological marker.

Keywords: autoantibodies, coeliac disease, epilepsy, immunoglobulins
To Arja
Acknowledgements

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Seinäjoki, March 2005

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<table>
<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine; serotonin</td>
</tr>
<tr>
<td>Ach</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>AGA</td>
<td>antigliadin antibody</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid</td>
</tr>
<tr>
<td>ANA</td>
<td>antinuclear antibodies</td>
</tr>
<tr>
<td>APC</td>
<td>antigen presenting cell</td>
</tr>
<tr>
<td>aPL</td>
<td>antiphospholipid</td>
</tr>
<tr>
<td>AMA</td>
<td>antimitochondrial antibody</td>
</tr>
<tr>
<td>β2-GPI</td>
<td>beta-2-glycoprotein I</td>
</tr>
<tr>
<td>CBZ</td>
<td>carbamazepine</td>
</tr>
<tr>
<td>CD</td>
<td>coeliac disease</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computerized tomography</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>EMA</td>
<td>endomysial antibody</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>glutamic acid decarboxylase</td>
</tr>
<tr>
<td>GluR</td>
<td>glutamate receptor</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>IDDM</td>
<td>insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>ILAE</td>
<td>The International League Against Epilepsy</td>
</tr>
<tr>
<td>IVIg</td>
<td>intravenous immunoglobulin</td>
</tr>
<tr>
<td>LTG</td>
<td>lamotrigine</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>OXC</td>
<td>oxcarbazepine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>PBC</td>
<td>primary biliary cirrhosis</td>
</tr>
<tr>
<td>PHT</td>
<td>phenytoin</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>RE</td>
<td>Rasmussen’s encephalitis</td>
</tr>
<tr>
<td>RR</td>
<td>risk ratio</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
</tr>
<tr>
<td>SMS</td>
<td>stiff man syndrome</td>
</tr>
<tr>
<td>VNS</td>
<td>vagus nerve stimulation</td>
</tr>
<tr>
<td>VPA</td>
<td>valproic acid</td>
</tr>
<tr>
<td>TCR</td>
<td>T-cell receptor</td>
</tr>
<tr>
<td>TLE</td>
<td>temporal lobe epilepsy</td>
</tr>
<tr>
<td>tTGA</td>
<td>tissue transglutaminase</td>
</tr>
</tbody>
</table>
List of original articles

The thesis is based on the following articles, referred to in the text by their Roman numerals:


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1 Introduction

Epilepsy is a common central nervous system disorder of considerable significance to public health. It is estimated, according to the Finnish Social Insurance Institution, that approximately 37000 people in Finland suffer from this condition (Social Insurance Institution 2003). The aetiology of epilepsy is variable and often unknown (cryptogenic/idiopathic). Various environmental and genetic factors contribute to the onset of epilepsy. Immunological mechanisms have also been implicated in the pathogenesis of epilepsy (Aarli 1993, Aarli 2000, Eeg-Olofsson 2003). Epilepsy is often purported to be associated with other diseases, often of suspected or proven autoimmune origin (Koppel 1997). Most of these diseases are associated with antibodies against autoantigens and the presence of these autoantibodies is often a hallmark of diagnosis in addition to clinical criteria (Reichlin 1998).

Catastrophic childhood epilepsies have responded to immunological therapy (Aarli 2000, Villani & Avanzini 2002) and there is recent evidence implicating antibodies against the glutamate receptor GluR3 in the pathogenesis of Rasmussen’s encephalitis (RE) (Rogers et al. 1994) and their presence in intractable seizures (Mantegazza et al. 2002) as well as in focal epilepsy (Wiendl et al. 2001). The response of RE to immunological treatments has been reported (Villani & Avanzini 2002). Antibodies against brain gangliosides have been described in patients with refractory temporal lobe epilepsy (TLE) (Giometto et al. 1998), and seizures have been shown to induce cytokine formation (Peltola et al. 2000a). Reports on the changes in immunoglobulin serum concentrations are some of the earliest concerning immunological changes in patients with epilepsy. Though often contradictory, most previous reports have implicated the role of the antiepileptic medication, especially phenytoin (PHT), in inducing these changes. Human leukocyte antigen (HLA) associations have also been reported in this context (Aarli 1993).

The prevalence of epilepsy has been reported to be increased in autoimmune disorders, especially in systemic lupus erythematosus (SLE) (Herranz et al. 1994; Toubi et al. 1995; Liou et al. 1996), in which epilepsy may precede clinical diagnosis of the autoimmune disorder for decades (Aarli 2000). An increased presence of anticardiolipin (aCL) and antinuclear antibodies (ANA) has been reported both in children (Angelini et al. 1994; Yoshimura et al. 2001; Eriksson et al. 2001) and adults with epilepsy (Verrot et al. 1997; Peltola et al. 2000b). Coeliac disease (CD) is a common autoimmune disease of the small
bowel associated with antibodies against wheat gluten and autoantibodies against small bowel antigens. In addition to small bowel biopsy, the presence of these antibodies is an essential part of the diagnosis of CD. The prevalence of epilepsy in patients with CD has been reported to be increased (Chapman et al. 1968) and CD has been reported to be more common in patients with epilepsy than in control populations (Cronin et al. 1998, Luostarinen et al. 2001).

We studied the presence of several immunological markers in a large cohort representing a general adult epilepsy population with predominantly well-controlled partial epilepsy in order to determine the frequency and associations of the markers with epilepsy attributes and co-morbidity. Possible associations between these immunological markers were also analysed.
2 Review of literature

2.1 Epilepsy

2.1.1 General aspects

Epilepsy is not a specific disease or even a single syndrome, but rather a broad category of symptom complexes arising from any number of disordered brain functions that themselves may be secondary to a variety of pathologic processes. Epilepsy is a group of conditions, the common and fundamental characteristics of which are recurrent, usually unprovoked seizures, which represent the clinical manifestations of excessive, synchronous, abnormal firing patterns of neurons that are located predominantly in the cerebral cortex (Engel & Pedley 1997). Hughlings Jackson was the first to define epileptic seizures as the result of occasional, sudden and excessive discharge of grey matter (Jackson 1873). The clinical manifestations are sudden and transient and can include a wide variety of motor, psychic and sensory phenomena, with or without alteration in consciousness or awareness. Thus, epilepsy is a condition in which the sufferer is prone to experience recurrent epileptic seizures (Shorvon 2000).

2.1.2 Occurrence

Epilepsy is the most common primary disorder of the brain. The prevalence of epilepsies has been assessed in numerous populations. Most studies in the United States, Europe and Asia have reported overall prevalences of 5 to 9 cases per 1000 persons (Annegers, 2001). Overall prevalence rates are similar in Europe, North America and Australia, around 7/1000; rates in children are slightly lower (Forsgren 2004). In Rochester, Minnesota, the prevalence of epilepsy was 6.66 cases per 1000 persons (Hauser et al. 1991) in a population of 1.7 million. Studies from less developed countries report larger differences in prevalence rates of active epilepsy (Placencia et al. 1992, Nicoletti et al. 1999,
The cumulative risk for epilepsy from birth through age 20 is about 1% and reaches 3% by age 75. Overall, about 3% of the population can be expected to have epilepsy at some time during their lives (Hauser 1997, Annegers 2001). In Rochester, Minnesota, the cumulative incidence of epilepsy by the age of 80 was 4% due to large number of elderly people in the population (Hauser et al. 1993). Incidence rates of epilepsy are typically 30–50 cases per 100000 person years. The reported rates are very high in the first year of life, decline through childhood and adolescence, plateau in middle life and rise sharply among the elderly (Annegers 2001). The incidence may be declining in children due to improved ante- and perinatal care (Annegers et al. 1995, Forsgren 2004). In Finland, with a population of approximately 5 million, 36535 persons received reimbursement for medical treatment of epilepsy in 2003 (Social Insurance Institution 2003).

### 2.1.3 Aetiology

The aetiology of epilepsy varies in different age-groups and geographical locations. Congenital and genetic conditions are the most common causes of epilepsy in childhood, while in older children and young adults, inherited disposition, hippocampal sclerosis, alcohol and drug abuse are important causes. In the elderly, vascular causes are common. Tumours and sporadic infection occur at all ages, but malignant tumours are more likely after the age of 30 (Shorvon 2000). In any acquired condition, epilepsy is more likely to occur if an inherited predisposition is present. In any individual case, several factors may predispose to epilepsy. Patients with genetic or chromosomal syndromes associated with epilepsy account for 2–3% of all cases of epilepsy (Beghi 2004). Known single gene disorders are estimated to underlie epilepsy in 1–2% of cases (Shorvon 2000). However, a genetic contribution is estimated to be present in up to 40% of patients with epilepsy comprising three major groups; mendelian disorders, non-mendelian or complex diseases, in which several loci together interact with environmental factors or by the maternal inheritance of mitochondrial DNA, and chromosomal disorders, in which a gross cytogenetic abnormality is present (Gardiner 2000).

### 2.1.4 Classification

#### 2.1.4.1 Classification of epileptic seizures

The present 1981 ILAE classification of seizures is presented in Table 1. It uses clinical form and EEG criteria in the classification and divides seizures into two categories, generalised and partial. This dichotomy in classifying seizure types depends on whether the seizure arises in a restricted part of the brain in one hemisphere or appears to involve both hemispheres from the onset. With advances in imaging and neurophysiology it has
become evident that some generalised seizures have underlying focal pathologies and some partial epilepsies originate in large neuronal networks (Shorvon 2000). Therefore, a new classification based solely on symptomatology has been proposed (Lüders et al. 1998).

**Table 1. ILAE classification of seizure type.**

<table>
<thead>
<tr>
<th>Partial (focal) seizures</th>
<th>Generalised seizures</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Simple partial seizures (consciousness not impaired)</td>
<td>1. With minor signs</td>
</tr>
<tr>
<td>(a) Focal motor without march</td>
<td>(b) With automatisms</td>
</tr>
<tr>
<td>(b) Focal motor with march (Jacksonian)</td>
<td>(a) With impairment of consciousness only</td>
</tr>
<tr>
<td>(c) Versive</td>
<td>(b) With automatisms</td>
</tr>
<tr>
<td>(d) Postural</td>
<td>C. Partial seizures evolving to secondary generalised seizures (may be generalised tonic-clonic, tonic or clonic)</td>
</tr>
<tr>
<td>(e) Phonatory (vocalization or arrest of speech)</td>
<td>1. Simple partial seizures (A) evolving to generalised seizures</td>
</tr>
<tr>
<td>2. With somatosensory or special-sensory symptoms</td>
<td>2. Complex partial seizures (B) evolving to generalised seizures</td>
</tr>
<tr>
<td>(Simple hallucinations, e.g., tingling, light flashes, buzzing)</td>
<td>3. Simple partial seizures evolving to complex partial seizures evolving to generalised seizures</td>
</tr>
<tr>
<td>(a) Somatosensory</td>
<td>(b) With automatisms</td>
</tr>
<tr>
<td>(b) Visual</td>
<td>B. Myoclonic seizures</td>
</tr>
<tr>
<td>(c) Auditory</td>
<td>Myoclonic jerks (single or multiple)</td>
</tr>
<tr>
<td>(d) Olfactory</td>
<td>C. Clonic seizures</td>
</tr>
<tr>
<td>(e) Gustatory</td>
<td>D. Tonic seizures</td>
</tr>
<tr>
<td>(f) Vertiginous</td>
<td>E. Tonic-clonic seizures</td>
</tr>
<tr>
<td>3. With autonomic symptoms or signs (including epigastric sensation, pallor, sweating, flushing, piloerection, and pupillary dilatation)</td>
<td>F. Atonic seizures (Astatic)</td>
</tr>
<tr>
<td>4. With psychic symptoms (disturbance of higher cerebral function); these symptoms rarely occur without impairment of consciousness and are much more commonly experienced as complex partial seizures</td>
<td>Combinations of the above may occur, e.g., B and F, B and D</td>
</tr>
<tr>
<td>(a) Dysphasic</td>
<td>May have:</td>
</tr>
<tr>
<td>(b) Dynamic (e.g., déjà vu)</td>
<td>Changes in tone that are more pronounced than in A.1</td>
</tr>
<tr>
<td>(c) Cognitive (e.g., dreamy states, distortions of time sense)</td>
<td>Onset and/or cessation that is not abrupt</td>
</tr>
<tr>
<td>(d) Affective (fear, anger, etc.)</td>
<td>B. Myoclonic seizures</td>
</tr>
<tr>
<td>(e) Illusions (e.g., macropsia)</td>
<td>Myoclonic jerks (single or multiple)</td>
</tr>
<tr>
<td>(f) Structured hallucinations (e.g., music, scenes)</td>
<td>C. Clonic seizures</td>
</tr>
<tr>
<td>B. Complex partial seizures (with impairment of consciousness; may sometimes begin with simple symptomatology)</td>
<td>D. Tonic seizures</td>
</tr>
<tr>
<td>1. Simple partial onset followed by impairment of consciousness</td>
<td>E. Tonic-clonic seizures</td>
</tr>
<tr>
<td>(a) With simple partial features (A.1-A.4)</td>
<td>F. Atonic seizures (Astatic)</td>
</tr>
<tr>
<td>followed by impaired consciousness</td>
<td>Combinations of the above may occur, e.g., B and F, B and D</td>
</tr>
</tbody>
</table>

**Partial seizures.** Partial seizures arise in specific often small loci of the cortex in one hemisphere. The expression of partial seizures requires the recruitment and synchronisation of a large cortical mass, however (Rogawski 2002). Partial seizures are divided into simple partial seizures, which occur without alteration of consciousness and complex partial seizures in which consciousness is impaired or lost. This is often difficult to assess, however. In a secondary generalised seizure, partial onset spreads to become a generalised attack (Dodson 2004). Partial seizures comprise motor, somatosensory or special sensory, autonomic and psychic manifestations.
Complex partial seizures, in their complete form, have three components; an aura is equivalent to simple partial seizure; altered consciousness in the form of memory loss and motor arrest and finally automatisms, which are involuntary motor actions. Complex partial seizures arise in 60% of cases from the temporal cortex and in about 30% of cases from the frontal lobe. Classification based on anatomic localisation is accommodated in the ILAE Classification of Epilepsies and Epileptic Syndromes due to the needs of epilepsy surgery. Temporal lobe seizures are divided into epilepsy arising from the mesial temporal lobe and from the lateral temporal neocortex. Other anatomic localisations of partial epilepsies are the frontal lobe, central (peri-rolandic) region and parietal and occipital lobes (Shorvon 2000).

Generalised seizures. According to the ILAE classification, generalised seizures are those in which the first clinical changes indicate initial involvement of both hemispheres (ILAE 1981). Consciousness is almost invariably impaired from the onset of the attack due to extensive cortical and subcortical involvement. Motor changes are bilateral and more or less symmetrical, and the EEG patterns are bilateral and grossly synchronous and symmetrical over both hemispheres (Shorvon 2000). Generalised seizures are believed to result from thalamocortical synchronisation (Avoli et al. 2001). In absence seizures, consciousness is lost and regained in an abrupt off-on pattern. Behaviour occurring at the onset may be perseverated, but more usually ceases instantly as the person begins to stare. The eyes may gaze straight ahead or deviate upward while the eyelids twitch faintly. A typical seizure lasts 5 seconds and rarely over 30 seconds.

Atypical absence seizures are more likely to be associated with the following features: longer duration, decreased postural tone and tonic activity. Patients more often have interictal abnormalities on EEG, multiple seizure types and mental retardation.

Myoclonus epilepsy refers to several progressive disorders in which either epileptic or non-epileptic myoclonus is a prominent feature. Myoclonic seizures occur in many different epileptic syndromes such as benign and severe myoclonic seizures of infancy, symptomatic epilepsies due to systemic storage diseases or defects in energy metabolism.

Clonic seizures are represented by repetitive, rhythmic jerking which is exemplified in tonic-clonic seizures. Tonic seizures are defined as rigid violent muscular contractions of axial and limb musculature which typically last for 30 s or less. Tonic seizures end abruptly with variable to no postictal symptoms. Isolated tonic seizures seem to be most common during sleep.

The tonic-clonic seizure is the formal name for a generalised convulsion, historically called a grand mal seizure. It is characterised by a sudden fall and dramatic, violent, involuntary shaking or muscular spasms of the limbs and body. Convulsions rarely last longer than 60 seconds. Longer lasting seizures tend to be prolonged. The postictal phase is characterised by diffuse hypotonia, slow deep respirations and unresponsiveness. Recovery over minutes to hours is marked by sleepiness, variable headache and complaints of musculoskeletal soreness upon awakening. Atonic seizures produce sudden reduction or loss of postural tone affecting posture to varying degrees. When extensive, patient may fall in the case of drop attacks or astatic seizures. (Dodson 2004)
2.1.4.2 Classification of the epilepsies and epileptic syndromes

Epilepsy is called symptomatic if the underlying cause is known, cryptogenic, if there is a presumed pathologic brain process which cannot be detected by present means, and idiopathic in the case of independent, intrinsic epilepsies. (Shorvon 2000) The revised ILAE classification of epilepsies and epileptic syndromes (ILAE 1989) is presented in Table 2. Juvenile myoclonic epilepsy, juvenile absence epilepsy, childhood absence epilepsy and epilepsy with generalised tonic-clonic seizures on awakening are the most common generalised epilepsies. On the other hand, temporal lobe epilepsies and frontal lobe epilepsies are the most common localisation related epilepsies. This classification is based on the anatomic origin of seizures.

Table 2. The ILAE Classification of Epilepsies and Epilepsy Syndromes.

<table>
<thead>
<tr>
<th>1 Generalised</th>
<th>2 Localisation related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic generalised epilepsies with age-related onset (in order of age)</td>
<td>Localisation-related epilepsies-idiopathic with age related onset</td>
</tr>
<tr>
<td>Benign neonatal familial convulsions</td>
<td>Benign neonatal convulsions</td>
</tr>
<tr>
<td>Benign neonatal convulsions</td>
<td>Benign myoclonic epilepsy in infancy</td>
</tr>
<tr>
<td>Childhood absence epilepsy</td>
<td>Juvenile absence epilepsy</td>
</tr>
<tr>
<td>Epilepsy with generalised tonic-clonic seizures on awakening</td>
<td>Other generalised idiopathic epilepsies not defined above</td>
</tr>
<tr>
<td>Epilepsies with seizures precipitated by specific modes of activation</td>
<td>Cryptogenic or symptomatic generalised epilepsies (in order of age)</td>
</tr>
<tr>
<td>West syndrome</td>
<td>Localisation-related epilepsies-symptomatic</td>
</tr>
<tr>
<td>Lennox-Gastaut syndrome</td>
<td>Epilepsia partialis continua</td>
</tr>
<tr>
<td>Epilepsy with myoclonic-astatic seizures</td>
<td>Syndromes characterised by specific modes of precipitation</td>
</tr>
<tr>
<td>Epilepsy with myoclonic absences</td>
<td>Temporal lobe epilepsies</td>
</tr>
<tr>
<td>Symptomatic generalised epilepsies</td>
<td>Central region epilepsies</td>
</tr>
<tr>
<td>Non-specific aetiology</td>
<td>Frontal lobe epilepsies</td>
</tr>
<tr>
<td>Early myoclonic encephalopathies</td>
<td>Parietal lobe epilepsies</td>
</tr>
<tr>
<td>Early infantile encephalopathy with burst suppression</td>
<td>Occipital lobe epilepsies</td>
</tr>
<tr>
<td>Other symptomatic epilepsies not defined above</td>
<td>Localisation-related epilepsies-cryptogenic</td>
</tr>
<tr>
<td>Epilepsies in other disease states</td>
<td>3 Epilepsies and syndromes undetermined as to whether focal or generalised</td>
</tr>
<tr>
<td>2 Localisation related</td>
<td>With both generalised and focal seizures</td>
</tr>
<tr>
<td>Localisation-related epilepsies-idiopathic with age related onset</td>
<td>Neonatal seizures</td>
</tr>
<tr>
<td>Benign epilepsy with centrotemporal spikes</td>
<td>Severe myoclonic epilepsy in infancy</td>
</tr>
<tr>
<td>Childhood epilepsy with occipital paroxysms</td>
<td>Electrical status epilepticus in slow wave sleep</td>
</tr>
<tr>
<td>Primary reading epilepsy</td>
<td>Acquired epileptic aphasia</td>
</tr>
<tr>
<td></td>
<td>Other undetermined epilepsies (not defined above) with unequivocal generalised or focal features</td>
</tr>
<tr>
<td></td>
<td>4 Special syndromes</td>
</tr>
<tr>
<td></td>
<td>Febrile convulsions</td>
</tr>
<tr>
<td></td>
<td>Isolated seizures or isolated status epilepticus</td>
</tr>
<tr>
<td></td>
<td>Seizures occurring only when there is an acute metabolic or toxic event caused by factors such as alcohol, drugs, eclampsia, non-ketotic hyperglycaemia</td>
</tr>
</tbody>
</table>
2.1.5 Treatment of epilepsy

2.1.5.1 Antiepileptic medications

General principles. Antiepileptic drugs act on one or more target molecules in the brain. These targets include ion channels, neurotransmitter transporters and neurotransmitter metabolizing enzymes. The ultimate effect of these interactions is to modify the bursting properties of neurons and reduce synchronisation in neuronal ensembles (Rogawski 2002). The mechanism of action of most antiepileptic drugs is not well understood. Many anticonvulsant drugs act through multiple complementary mechanisms and this may explain why anticonvulsants which target critical brain excitability systems protect against seizure activity with relatively low central nervous system toxicity (Rogawski 1998).

Antiepileptic drug actions can be categorised into those that involve (a) modulation of voltage-dependent ion channels, (b) enhancement of synaptic inhibition, and (c) inhibition of synaptic excitation. Modulation of voltage-dependent ion channels (sodium, calcium, potassium) is integral in regulation of seizure discharges and they are critical elements in neurotransmitter release and modify excitatory and inhibitory transmissions. According to animal models, anticonvulsant action can be achieved by blockade of sodium or calcium channels, and probably through facilitation of potassium channels, as well as by enhancing the inhibition mediated by GABA_\_A receptors or through effects on the glycine system, the regionally specific transmitter systems (including monoamines such as catecholamines, serotonin and histamine, and neuropeptides, including opioid peptides and neuropeptide Y), and the inhibitory neuromodulator adenosine. In addition, blockade of excitatory amino receptors (including NMDA, AMPA, metabotrobic and possibly kainate types) can protect against seizures. (Rogawski 2002) The molecular targets and clinical efficacy of antiepileptic drugs are presented in Table 3.
Table 3. Molecular targets and clinical efficacy of antiepileptic drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Na⁺ Channels</th>
<th>Ca²⁺ Channels</th>
<th>GABA_A Receptor</th>
<th>GABA_A Transporter</th>
<th>GABA_B Receptor</th>
<th>NMDA Receptor</th>
<th>Clinical Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial, GTC</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial, GTC</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial, GTC</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial, GTC, absence</td>
</tr>
<tr>
<td>Zonisamide⁺</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial, GTC, myoclonic</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Absence</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial</td>
</tr>
<tr>
<td>Benzo-diazepines</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Broad spectrum</td>
</tr>
<tr>
<td>Vigabatrin</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial</td>
</tr>
<tr>
<td>Tiagabine</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>Partial</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Partial</td>
</tr>
<tr>
<td>Felbamate</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>Broad spectrum</td>
</tr>
<tr>
<td>Topiramate⁺</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Broad spectrum</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Broad spectrum</td>
</tr>
</tbody>
</table>

*Zonisamide and topiramate are weak carbonic anhydrase inhibitors

⁺Vigabatrin, lamotrigine, zonisamide and topiramate may be useful in treating infantile spasms

GTC Generalised tonic-clonic

Modified from Rogawski 2002

Carbamazepine and oxcarbazepine. Carbamazepine (CBZ) and oxcarbazepine (OXC) are structurally related to imipramine (MacDonald 2002a, Bialer 2002). These tricyclic compounds share some common pharmacological characteristics. Both CBZ and OXC are sodium channel blockers, but OXC and its monohydroxy derivative limit the firing of sodium-dependent action potentials at lower concentrations than CBZ. They also differ in their effect on calcium channels and may differ in their effect on extracellular 5-HT and blockage of Ach receptors. Both block NMDA-receptors. As a whole, the mechanistic differences between CBZ and OXC are subtle (Macdonald 2002a, McLean 2002, Sillanpää 2004). However, OXC induces hepatic enzymes less than CBZ and has fewer drug interactions. OXC is associated with benign hyponatremia more often than CBZ in most cases.

CBZ has been used in epilepsy treatment from the 1960s (Loiseau 2002). It is the drug of choice in partial and secondary generalised seizures and it is also useful in generalised...
tonic clonic seizures associated with idiopathic generalised epilepsy. In children its use in
genralised epilepsy is more controversial. It can exacerbate the non-convulsive seizures
of the Lennox-Gastaut syndrome and it aggravates generalised absence and myoclonic
seizures in idiopathic epilepsy while often controlling the tonic-clonic seizures (Shorvon
2000). Febrile convulsions are resistant to CBZ.

The indications for OXC are the same as for CBZ, although OXC is better tolerated
than CBZ (Shorvon 2000, Sillanpää 2004).

Valproic acid. Valproate (VPA), synthesised in 1882 by Burton (Burton 1882), has
become the mainstay therapy against generalised seizures. Its antiepileptic efficacy was
discovered in 1962 by Eymard (Meunier et al. 1963), but its mode of action is not fully
understood. VPA enhances GABA function at high concentrations. VPA also potentiates
GABA-mediated postsynaptic inhibition, inhibits GABA degrading enzymes and may
increase the synthesis of GABA by increasing GAD activity. It also reduces excitatory
transmission and reduces the threshold for calcium and potassium conductance. VPA has
an inhibitory effect on voltage dependent sodium channels, but this most probably does
not contribute to its anticonvulsive properties (Rogawski 2002, Löscher 2002).

VPA has activity against a wide range of seizure types. It is the drug of choice in pri-
mary generalised epilepsy treating the generalised absence, myoclonus and tonic-clonic
seizures in primary generalised epilepsy syndromes. It is also useful in atypical absence
and atonic seizures in the Lennox-Gastaut syndrome, for which it is the drug of choice. It
is also effective in partial and secondary generalised seizures, although CBZ and OXC are
usually the first-line therapies (Shorvon 2000).

The use of VPA has been overshadowed in recent years by reports on endocrine
side-effects in various populations in both genders and in animal models (Genton &
Gelisse 2002). Weight gain is one the most important side-effects of VPA due to insulin
resistance and may be associated with anovulatory cycles, amenorrhea and polycystic
ovary syndrome, which may be reversible (Isojärvi 1993, 1996, 1998, Mikkonen et al.
2004). Other main disadvantages are cognitive effects, tremor and hair loss, and in
children a risk for severe hepatic and pancreatic disturbances also exists (Arroyo 2004). A
consensus has been reached in the clinical guidelines for VPA prescription in women with
epilepsy (Bauer et al. 2002).

Phenytoin. Merritt and Putnam demonstrated the antiepileptic efficacy of PHT in 1937
and 1938 (Putnam & Merritt 1937). The major anticonvulsant mechanism of PHT is
believed to be an effect on the sodium channels. It also affects sodium-potassium
adenosine triphosphatase activity, various enzyme systems, synaptic transmission due to
an inhibiting effect on calcium, posttetanic potentiation, neurotransmitter release and
cyclic nucleotide metabolism (DeLorenzo & Sun 2002). PHT is one of the most com-
monly used antiepileptic drugs in the world and remains the most commonly used AED in
North America (Wilder & Bruni 2002).

PHT is as effective as any other first-line drug in partial and in tonic-clonic seizures
especially when they are secondary generalised (Shorvon 2000). Among the common and
important side-effects is CNS toxicity. Adverse effects also commonly involve other sys-
tems: hematopoietic, gastrointestinal, immune, endocrine, skeletal and skin as well as
reduced production of IgA in some patients (Slavin et al. 1974, Bardana et al. 1983, Ruff
1987). PHT is probably the antiepileptic drug with the most problematic interaction pro-
file due to its saturable kinetics (Shorvon 2000). It is also highly protein bound and meta-
bolized by hepatic P450 enzymes. Due to long term side-effects, interaction profile and availability of newer sodium channel blockers clinical usage of PHT has rapidly diminished in Finland and other West European countries.

Lamotrigine. Studies on lamotrigine (LTG) began in the 1970s and in human studies of epilepsy in the mid 1980s. LTG is structurally unrelated to any other antiepileptic drug. Its effects on sodium and calcium channels are mechanistically similar to those of PHT. PHT affects release of glutamate and γ-amino butyric acid (GABA) at similar concentrations, but LTG is twice as effective in inhibiting glutamate release compared with GABA release. In experimental seizure models, LTG has a profile of action rather similar to that of PHT and CBZ. It is useful as a second-line therapy for primary generalised epilepsy or partial and secondary generalised seizures. It is licensed for monotherapy and is generally well-tolerated (Shorvon 2000, Stephen & Brodie 2002).

Other antiepileptic drugs. There is a plethora of older and newer antiepileptic drugs used primarily against partial seizures. Phenobarbital has been used since 1912 and primidone from the 1950s, but they are no longer deemed clinically useful in Finnish practice due to the considerable CNS and long term adverse effects. Ethosuximide and acetazolamide were also introduced in the 1950s. Ethosuximide inhibits low-threshold calcium currents in the thalamus and is effective in generalised absence seizures (Holland & Ferrendelli 2002, Sherwin 2002). Acetazolamide inhibits carbonic anhydrase and is effective in tonic-clonic seizures, in cases of juvenile myoclonus epilepsy, in absences, as an adjunctive in partial epilepsy and in catameneal epilepsy, but development of tolerance necessitates drug withdrawal periods (Neufeld 2004). ACTH has been used in the treatment of infantile spasms and status epilepticus. Its mode of action is unknown (Meierkord 2004).

The newer drugs are licensed mainly as an adjunctive therapy in partial seizures. Benzodiazepines affect the BZD binding site on the GABA-receptor (Macdonald 2002b). Clonazepam is relatively sedative and used as second-line therapy against partial and generalised seizures (Sato & Boudreau 2004). Diazepam, lorazepam, midazolam and nitrazepam are other BZDs that are used in clinical practice against seizures (Greenfield & Rosenberg 2004). Clobazam is indicated for adjunctive therapy for refractory partial seizures and generalised seizures. It is better tolerated than other BZDs but tolerance develops in 50% of cases (Schmidt 2002, Dalby 2004).

The newer GABAergic drugs vigabatrin and tiagabine have different modes of action. Vigabatrin is a close structural analogue of GABA and binds irreversibly to GABA transaminase and causes a large increase in the extracellular GABA concentration. It has a potential for severe neuropsychiatric side-effects, and, in up to 50% of cases, may cause a peripheral visual field defect, which may be irreversible (Kälviäinen et al. 1998, Ben-Menachem 2002). For these reasons, vigabatrin is used only in selected cases. Tiagabine inhibits the GABA-transporter-1 reversibly causing a small increase in extracellular GABA concentration (Giardina 2002). It is suitable as add-on therapy in partial epilepsy, especially when deterioration in cognitive performance is undesirable (Kälviäinen 2002).

Gabapentin interacts with a specific high-affinity binding site in brain membranes that is an auxiliary protein subunit of voltage dependent calcium channels. It also reduces the release of several neurotransmitters (Taylor 2002, Browne 2004). Pregabalin is a new GABA analogue with the same clinical profile as gabapentin (Ben-Menachem & Kugler 2004). Felbamate blocks the NMDA receptor. It is used in severe partial epilepsy and
Lennox-Gastaut syndrome, but severe hepatic failure and aplastic anemia have considerably limited the usefulness of felbamate in clinical practice. (Pellock et al. 2002)

**Topiramate** is derivative of D-fructose. It enhances GABA-action, but inhibits sodium conduction, AMPA subtype glutamate receptors and weakly inhibits carbonic anhydrase (White 2002). It is a potent wide-spectrum antiepileptic drug possibly due to its multiple modes of action (Privitera & Twyman 2002). **Levetiracetam** binds to a specific receptor in the brain and modulates high-voltage calcium currents, suppresses Zn$^{2+}$ allosteric modulation of GABA and glycine-gated currents and delays secondary action potentials. Its mode of action differs from those of other antiepileptic drugs (Lynch et al. 2004). Levetiracetam is well-tolerated and indicated in partial seizures, but may be also effective in generalised epilepsies (Leppik 2002).

The role of new antiepileptic drugs in the treatment of new onset epilepsy and refractory seizures has been recently evaluated by the American Academy of Neurology and the American Epilepsy Society. According to the recommendation, treatment of newly diagnosed epilepsy can be initiated with lamotrigine, gabapentin, oxcarbazepine or topiramate (French et al. 2004a). In refractory partial epilepsy, the use of gabapentin, lamotrigine, tiagabine, topiramate, oxcarbazepine, levetiracetam, and zonisamide as add-on therapy is recommended. Oxcarbazepine and topiramate can be used as monotherapy against refractory partial seizures. Topiramate can also be used for the treatment of generalised tonic-clonic seizures. (French et al. 2004b)

### 2.1.5.2 Epilepsy surgery

The goal of the presurgical evaluation of epilepsy is to delineate the epileptogenic zone. Advances in neuroimaging have revolutionised the prospects of epilepsy surgery and surgery can be curative in many cases. In a recent cohort of Finnish patients with unilateral temporal lobe epilepsy undergoing surgery after introduction of a standardized MRI protocol, 52% became seizure free (Jutila et al. 2002). The first resective surgery was performed in 1880 and the development of the EEG in the late 1930s ushered in a period of intensive development. The first temporal lobectomy was performed in the late 1940s and the standard operation with the removal of the hippocampus was introduced in 1951. It has been estimated that between 2 and 5% of patients with medically refractory epilepsy would benefit from epilepsy surgery. The main types of surgical approach are presented in Table 4.
Table 4. The main types of surgical approach in epilepsy surgery (Shorvon 2000).

<table>
<thead>
<tr>
<th>Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Focal resection of hippocampal sclerosis including temporal resection and more selective operations.</td>
</tr>
<tr>
<td>2. Focal resection of other lesions (lesionectomies) including developmental abnormalities, neoplasms, vascular abnormalities, ischemic lesions, mesial temporal sclerosis, inflammatory diseases, posttraumatic gliosis and hemosiderin deposition and non-specific gliosis.</td>
</tr>
<tr>
<td>3. Non-lesional focal resections.</td>
</tr>
<tr>
<td>4. Multilobar resections.</td>
</tr>
<tr>
<td>5. Functional procedures such as multiple subpial transection and corpus callosectomy</td>
</tr>
</tbody>
</table>

2.1.5.3 Other treatments

The ketogenic diet was introduced in 1921 (Geyelin 1921). It is a high-fat, low-carbohydrate and low-protein regimen that increases the production of ketone bodies. It has broad anticonvulsive properties and possibly antiepileptogenic activity. It may favourably affect cerebral energetics and increase GABA shunt activity and thus increase resistance to seizures. It is applied in a hospital environment due to the risk of severe metabolic acidosis. The main indications are medically refractory seizures in childhood. (Shorvon 2000, Nordli & Vivo 2001, Kossof & Vining 2004)

Left vagus nerve stimulation (VNS) was approved 1997 in the USA as an adjunctive therapy for adults and adolescents over 12 years of age whose partial onset seizures are refractory to antiepileptic medications (Schachter 2004). Electrical stimulation of the peripheral vagus nerve requires polysynaptic transmission to mediate the anti-seizure effect. Activation of vagal visceral afferents which have diffuse CNS projections broadly affects neuronal excitability. VNS exerts both acute and long term antiepileptic effects, however, the role of VNS has not been fully established (Shorvon 2000).

2.2 The immune system and autoimmunity

2.2.1 General aspects

The physiological functions of the immune system are engaged in defence against foreign infectious agents: parasites, bacteria and viruses. It also defends the body from toxic agents and maintains the antigenic homeostasis in the body. It does this by eliminating cells perceived by the immune system to be foreign. Deviation in the immune response can harm tissue structures in the form of autoimmune diseases. To meet the aforementioned demands, the immune system has evolved into two parts: one responsible for immediate, relatively generic action against external agents, and another system that responds specifically to external threat. The innate immune system responds immediately and the
adaptive or acquired immune system responds specifically. Both are functionally intertwined (Birnbaum 1998).

2.2.2 The innate immune system

The innate immune system recognises carbohydrate structures, especially those on the surfaces of bacteria. These carbohydrate moieties differ from those present on host cells and are recognised by proteins some of which are soluble and others are present on cell surfaces. Immune interaction with these carbohydrates induces secretion of cytokines and activates complement. Cells participating in the innate immune system are polymorphonuclear leukocytes, macrophages, dendritic cells, natural killer cells and T cells. Granulocytes circulate predominantly in the blood and macrophages in different tissues. They recognise bacterial structures. Pathogens are destroyed in these cells by proteases, reactive oxygen metabolites and nitrous oxide radicals. Natural killer cells recognise and destroy cells infected by certain viruses and parasites. Due to evolution of the defence mechanisms of pathogens, the human body has developed the adaptive immune system. (Birnbaum 1998, Pette et al. 1999)

2.2.3 The adaptive immune system

2.2.3.1 Lymphocytes

T and B lymphocytes derived from primary lymphoid tissue (the bone marrow, fetal liver and spleen) are the main cell components of the adaptive immune system.

The majority of cells destined to become T-cells migrate to the thymus where induction of genes leads to the expression of each T cell’s unique antigen receptor (TCR). That is followed by positive and negative selection of T cells based on their antigen specificities. The antigenic specificity of the TCR is determined by amino acids in the antigen-binding cleft. (Birnbaum 1998)

B lymphocytes originate in primary lymphoid tissue and then migrate to secondary lymphoid organs (spleen, lymph nodes, gut-associated lymphoid tissue). There, after contact with antigens, which can interact with immunoglobulin receptors, they differentiate further. The unique property of B cells is their ability to secrete antibodies or immunoglobulins. Antibody genes continually change as B cells encounter antigen and proliferate. Continuous mutations occur in the genes coding for the variable regions of Igs. Antibody concentrations increase and their affinity increases after contact with antigen. B cells act as highly efficient antigen presenting cells (APCs). They bind antigen, digest it and re-express the digested peptides on their cell surface in the context of major histocompatibility complex (MHC) proteins. (Birnbaum 1998; Pette et al. 1999)

Immunoglobulin isotypes are presented in Table 5.
2.2.3.2 Natural killer cells, macrophages and dendritic cells

Natural killer (NK) cells are bone-marrow-derived cells which arise from the same precursors as T cells (Poggi & Mingari 1995). NK cells occur in the absence of immune challenge and kill e.g. tumour cells carrying certain target molecules.

Macrophages are bone marrow-derived cells with multiple functions. They are not antigen specific but they can, especially when activated, express cell surface proteins coded for by the genes of the MHC. These MHC proteins have grooves into which peptides can fit. Macrophages present peptide-antigen complexes on the cell surface after ingestion and proteolysis, a process called antigen processing. Activated macrophages secrete cytokines, which modulate T cell, B cell and macrophage function. Interleukin (IL)-1, tumour necrosis factor (TNF)-α, and prostaglandins are also secreted by macrophages. (Birnbaum 1998)

Dendritic cells are pleomorphic populations of cells originating in the bone marrow. Mature dendritic cells act as APCs. Dendritic cells are present on body surfaces, where they encounter foreign antigens and thereafter migrate to areas of lymphoid cell accumulation and maturation such as the thymus, lymph nodes and Peyer’s patches (Steinman et al. 1995). There they affect T lymphocyte selection and activate T and B cells (Szakal & Tew 1992).

2.2.4 Major Histocompatibility Complex

The MHC is a complex of genes present on chromosome 6. They play roles in the immune system and also in somatic cell differentiation (Salter-Cid & Flajnik 1995). Certain MHC phenotypes are associated with autoimmune diseases (Nepom 1993).

There are three major classes of MHC genes, class I, class II and class III genes.

<table>
<thead>
<tr>
<th>Isotype</th>
<th>Molecular weight (kD)</th>
<th>Form</th>
<th>Serum concentration (g/L)</th>
<th>Half-time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA1</td>
<td>160</td>
<td>mono/dimer</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>IgA2</td>
<td>160</td>
<td>mono/dimer</td>
<td>0.5</td>
<td>7</td>
</tr>
<tr>
<td>IgD</td>
<td>180</td>
<td>monomer</td>
<td>0.3</td>
<td>3</td>
</tr>
<tr>
<td>IgE</td>
<td>190</td>
<td>monomer</td>
<td>0.0001</td>
<td>3</td>
</tr>
<tr>
<td>IgG1</td>
<td>150</td>
<td>monomer</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>IgG2</td>
<td>150</td>
<td>monomer</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>IgG3</td>
<td>170</td>
<td>monomer</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>IgG4</td>
<td>150</td>
<td>monomer</td>
<td>0.5</td>
<td>21</td>
</tr>
<tr>
<td>IgM</td>
<td>900</td>
<td>pentamer</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

kD: Kilodalton
Modified from Pette et al. 1999
Class I and class II MHC proteins are required for antigen recognition by T cells. Class III proteins are part of the complement system. Class I MHC gene products are expressed on almost all somatic cells. Under normal conditions the products of class II genes are expressed almost exclusively on the surfaces of bone marrow–derived cells such as macrophages and B cells. In inflammatory areas cytokines can induce other cells to express class II MHC proteins. This phenomenon may be important for the development of autoimmune diseases. If a particular MHC protein is unable to bind and present a particular peptide, that peptide is invisible to the immune system. (Birnbaum 1998)

2.2.4.1 Initiation of adaptive immune response

The TCR interact with short peptides processed by antigen processing cells (APC)(Germain 1995). T cells are selected in the thymus based on a modest degree of responsiveness to self proteins. Most T cells respond therefore to their own constellation of MHC molecules, although they are restricted in their response. Cell surface proteins on T cells insure the specificity of their interactions with APC. CD4+ proteins, present on a subpopulation of T cells, react with class II MHC molecules while T cells which express CD8+ proteins react with class I MHC proteins. CD4+ cells are usually helper cells, which assist B cells. CD8+ T cells are mostly cytotoxic. In addition to the class I and II MHC protein CD4+/CD8+ interactions, the presence of adhesion molecules and co-stimulatory molecules is required (Finkelman et al. 1992). If an antigen is presented by non-professional cells such as astrocytes, co-stimulation is absent. This results in long-lasting unresponsiveness known as anergy (Johnson & Jenkins 1994). Anergic T cells do not produce IL-2. Anergy is one of the major controlling mechanisms of the immune system.

Cytokines are biologically active polypeptides secreted by a variety of cell types including fibroblasts, astrocytes, macrophages and T cells. They can activate or suppress the immune system and therefore some are pro-inflammatory while others are anti-inflammatory (Belardelli 1995, Karnitz & Abraham 1995).

CD4+ T cells (helper cells) can be divided to Th 1 and Th 2 cells. Th1 cells secrete INF-γ and IL-2. Th 1 cells also participate in delayed hypersensitivity reactions and stimulate the differentiation of CD8+ cells into mature cytotoxic cells. Th 2 cells and the cytokines which they secrete are necessary for B cell differentiation into mature antibody secreting plasma cells. In general, cytokines produced by Th 1 and Th 2 cells have antagonist effects.

A multitude of genes are involved in shaping the pattern of immune response. Among them are genes encoding the TCR and the MHC, those involved in the regulation of cytokine secretion and those associated with proteins involved in antigen processing (Birnbaum 1998).
2.2.4.2 Immune tolerance

The recognition of self and non-self antigens is imperative to the immune system in order to prevent destructive responses to self-antigens. Disturbances in these mechanisms may lead to autoimmune disorders. Key elements of self-tolerance are T and B cell regulation.

The most important T cell regulatory mechanisms are: 1. Intrathymic clonal deletion: Cells with TCRs of too high or low affinity for self-antigens are eliminated in the thymus. 2. Peripheral clonal deletion: All autologous antigens are not expressed in the thymus. One mechanism to eliminate autoreactive cells is exposure to high doses of antigen (Critchfield et al. 1994), which results in apoptosis of the stimulated cells. 3. Peripheral clonal unresponsiveness: If T cells encounter antigens without costimulatory signals, they become anergic (Johnson & Jenkins 1994). This is the case, if the APC is a non-professional cell. Anergy can be reversed in the presence of professional APCs and high concentrations of cytokines as in inflammatory areas. Infections and traumas such as surgery can therefore induce autoimmune processes. 4. Clonal ignorance: In some cases, expression of antigens by cells that are not immunologically competent can result in inactivation of T cells. These T cells are not anergic and in other circumstances react with the same antigen. 5. Regulatory networks: TCRs and antibodies can function as stimulators of immune responses. If responses are directed against the antigen binding portions of these receptors (the idiotypes), they are called anti-idiotypic and can suppress or augment the effects of idiotype-expressing T cells and antibodies (Beraud 1991). 6. Immune deviation: The site of antigen presentation and the amount of antigen determine the immune response. In oral tolerance, ingestion of antigen and the exposure of gut-associated lymphoid tissue results in suppression of T-cell mediated immune responses.

Similar mechanisms also exist for B cells, such as bone marrow clonal deletion, peripheral clonal unresponsiveness and peripheral clonal deletion. In addition, there are B cell-specific mechanisms of regulation. 1. Maturational arrest: Normal expression of auto-antigens can prevent the maturation of autoreactive B-cells. 2. Follicular exclusion: Autoreactive B cells, especially after having captured an antigen, are excluded from entering secondary lymphoid follicles (Birnbaum 1998). This results in apoptosis (Cyster & Goodnow 1995).

2.2.5 Autoimmune diseases

2.2.5.1 General aspects

Different criteria have been proposed for defining an autoimmune disease. Originally clinical and laboratory criteria were proposed for defining autoimmune diseases, e.g. hypergammaglobulinemia, high levels of IgG autoantibodies, lymphoid cell aggregates in affected tissues, antigen-antibody complex deposits in affected tissues, clustering with other autoimmune diseases due to common genetic susceptibility factors, and benefit from corticosteroid or immunosuppressive treatments were used as criteria for diagnosis
of several autoimmune diseases (Mackay & Burnet 1963). These criteria have been considered too non-specific for scientific work, however (Rose & Mackay 1998).

Rose and Bona focused on causality, whether autoimmunity is the cause of the human disease or a consequence or harmless accompaniment (Rose & Bona 1993). Evidence can be divided into direct, indirect or circumstantial. Direct evidence requires transmissibility from human to human or human to animal. This is possible only with diseases mediated with autoantibodies, such as myasthenia gravis. The effects of the autoantibody can also be displayed in vitro to show the characteristic functional deficits of the disease. Indirect evidence is applied in animal models, where a host antigen analogous to the putative autoantigen in the human disease is used. Circumstantial evidence includes the presence of high affinity or clonally limited autoantibodies, a clonally limited cellular immune response as determined by the TCR variable region gene or MHC class II restriction, genetic and familial clustering, and the ability to induce an unresponsive state with the putative autoantigen (Rose & Mackay 1998).

In the U.S., about 1 in 31 Americans or 8.5 million people are afflicted with various autoimmune disorders. The diseases with the highest prevalence rates are Graves’ disease, insulin dependent diabetes mellitus (IDDM), pernicious anemia, rheumatoid arthritis (RA) and thyreoiditis, which make up over 90% of the total number of cases. Glomerulonephritis, multiple sclerosis, lupus erythematosus (SLE) and Sjögren’s syndrome make up the majority of the remaining cases, the others being rather rare. Women were at 2.7 times greater risk than men to develop autoimmune disease (Jacobson et al. 1997). In Finland, the prevalence of RA is 0.8% with approximately 40000 patients (Kaipiainen-Seppänen 2004). The prevalence of MS in Finland is 0.1% and in high risk areas 0.2% (Sumelahti et al. 2003) with a total patient population of approximately 5000 patients. The prevalence of SLE is 0.028% in the Finnish population with 1500 patients (Helve 1985).

2.2.5.2 Genetic and environmental factors

All the autoimmune disorders represent a violation of the prospects of self-tolerance. The responsiveness and unresponsiveness to autologous antigens do not differ from the response to foreign antigens; the same rules apply. The immune system responds to antigenic stimuli in a stereotypic manner dependent on the properties of the antigen, the genetics and previous experience of the host, and the circumstances of antigen presentation; it is blinded to self versus non-self. Genetic factors contribute about one-third to one-half of the risk for most autoimmune diseases. Both MHC and non-MCH genes contribute to the disposition of autoimmune disease (Kwok WW & Nepom GT 1998, Vyse et al. 1998).

Familial clustering has been reported in many autoimmune disorders, e.g. in MS (Kinnunen et al. 1984, Tienari et al. 1992) and SLE (Koskenmies et al. 2004) in the Finnish population. In some subjects the cumulative load of genetic risk is such that the individual is at all times "on the brink" of autoimmunity; in others the genetic risk is so weak that a potent environmental stimulus is needed. Environmental processes probably have an effect in virtually all human autoimmune diseases (Rosen & Casciola-Rosen 1998). Historically infectious agents have been most cited as triggers and recent work has centred on the molecular mimicry hypothesis (Fujinami 1998, Moudgil & Sercarz 1998). However, a
clear-cut example of a human autoimmune disease produced by molecular mimicry are missing.

Examples of environmental mechanisms are ultraviolet radiation, which can expose intracellular epitopes through apoptosis; environmental pollutants, which may induce changes in nucleolar constituents; and medicinal drugs that induce lupus by promoting hypomethylation of DNA. Common threads uniting the autoimmune disorders are the presence of an autoimmune response based on cumulative genetic risk factors combined with an environmental contribution (Rose & Mackay 1998).

2.3 Epilepsy and autoimmunity

2.3.1 General aspects

The evidence for the involvement of autoimmune mechanisms in some forms of epilepsy is based on three main areas: the childhood epilepsy syndromes, epilepsy associated with immunologically mediated diseases, and the more common unselected groups of patients with epilepsy (Palace & Lang 2000). The effect of immunological treatments in patients with epilepsy is documented mostly in children with catastrophic epilepsy syndromes. Immunological sequelae of treatment with antiepileptic drugs have been reported to be associated with many drugs (Ponti et al. 1993, Knowles et al. 1998).

2.3.1.1 Childhood epilepsy syndromes

Rasmussen’s encephalitis (RE) is a rare progressive disorder of unilateral brain dysfunction (Rasmussen 1958). It is characterised by intractable focal seizures and inflammatory histopathology with perivascular lymphocyte cuffing and scattered microglial nodules (Anderman 1991). It commences usually in middle childhood and leads to hemiparesis and mental retardation. Case reports with some effect of intravenous immunoglobulins (IVIg), corticosteroids and plasmapheresis have emerged. Rabbits immunised with a fusion protein of the glutamate receptor (GluR) 3 developed seizures and histopathological changes mimicking those of RE patients. Some patients with RE have antibodies against GluR3 (Rogers et al. 1994, Mantegazza et al. 2002), but these antibodies are present also in focal epilepsy (Wiendl 2001). Antibodies against GluR3 could activate cortical neurons and induce cytotoxicity. The unilateral histopathology of human RE is not present in experimental animals, though (Aarli 2000).

Landau-Kleffner syndrome is characterised by aphasia, behavioural problems and seizures, which are often of the partial motor type. Autoantibodies directed against brain endothelial cells and neuronal nuclear proteins have been reported (Connolly et al. 1999). Some case reports show a beneficial effect of IVIg (Lagae et al. 1988, Mikati et al. 1998),
and improvement of language functions after immunomodulatory treatment has also been reported by some, but not all researchers.

West’s syndrome and Lennox-Gastaut syndromes have very different clinical phenotypes, but both have been reported to respond to IVIg therapy (Echenne et al. 1991, Van Engelen et al. 1994, Duse et al. 1996).

2.3.1.2 Epilepsy associated with autoimmune disorders

The incidence of epilepsy in patients with systemic lupus erythematosus (SLE) is between 10% and 20% at some stage of the disease. This is 8 times the prevalence of epilepsy in the general population (Aarli 2000). In 5% to 10% of patients, the onset of seizures precedes the onset of SLE by several years (Mackworth-Young & Hughes 1985, Toubi et al. 1995, Glanz et al. 1998). This could mean that antiepileptic drugs precipitate SLE or that epilepsy and SLE occur together as a manifestation of a genetically determined predisposition. According to Mackworth-Young and Hughes, epileptic seizures occurring before the manifestation of SLE were more often primary generalised, but seizures occurring after the onset of SLE were either focal or generalised tonic-clonic (Mackworth-Young & Hughes 1985). Seizures may occur in SLE patients based on immune-mediated neuronal damage, because of thrombotic events in cortical blood vessels, or they may be secondary to hypertensive encephalopathy or renal failure (Aarli 2000). Epilepsy is particularly common in association with the presence of antiphospholipid antibodies (aPL) (Herranz et al. 1994, Toubi et al. 1995, Liou et al. 1996). Contradictory reports exist, however (Sachse et al. 1995, Formiga et al. 1997).

aPL have been shown to depolarise synaptoneurosomes from the rat brainstem and this has been postulated to be an additional mechanism in nonthromboembolic CNS manifestations (Chapman et al. 1999). IgG antibodies to cardiolipin have been found in 30% to 60% of unselected patients with SLE (Toubi et al. 1995, Sachse et al. 1995). aPL present in the sera of SLE patients are heterogenous and the different specificities may cause different clinical manifestations (Rand 1989). Anti-B2-glycoprotein I antibodies may have a direct pathogenic role in thrombosis (Sachse et al. 1995, Day et al. 1998). The presence of aPL in CSF has been longitudinally associated with clinical symptoms (Yeh et al. 1994). In some groups of patients aPL have been reported in association with strokes and abnormal imaging findings (Sabet et al. 1998). On the other hand, abnormal MRI findings have been associated with the presence of other vascular disease risk factors and not with aPL alone (Hachulla et al. 1998). Antiganglioside antibodies have been found in SLE patients as well, and antibodies have been reported to be epileptogenic (Galeazzi et al. 2000).

Stiff man syndrome (SMS) is a rare CNS syndrome characterised by progressive rigidity and painful spasms of the muscles (Solimena et al. 1988, Meinck 2001). Serum antibodies to glutamic acid decarboxylase (GAD) have been detected in 63% of patients with stiff man syndrome. GAD catalyses the conversion of glutamate to GABA. This enzyme is concentrated within GABAergic nerve terminals and pancreatic B cells. Serum containing anti-GAD antibodies binds to pancreatic B cells in 95% of patients, whereas CSF antibodies are present in 80% of patients.
The prevalence of epilepsy in SMS is reported to be 12% (Palace & Lang 2000). Theoretically both seizures as well as other neurological manifestations of this disorder can be attributed to the interference with the inhibitory neurotransmitter GABA. This is supported by the clinical response to GABA agonists such as benzodiazepine, and in some cases, corticosteroids and plasmapheresis (Vicari et al. 1989, Brashear & Phillips 1991, Solimena & Camilli 1991).

Hashimoto’s encephalopathy is often associated with seizures, confusion and hallucinations (Shaw et al. 1991, Henchey et al. 1995). Antithyroid antibodies are always present. It responds to corticosteroids and CSF antibodies are present. A common brain/thyroid antibody has been suggested to explain the syndrome (Palace & Lang 2000).

### 2.3.1.3 Antibodies associated with unselected patients with epilepsy

A summary of antibodies reported in epilepsy populations is presented in Table 6.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Antibody target</th>
<th>Immunotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rasmussen’s encephalitis</td>
<td>GluR3</td>
<td>Plasma exchange or protein A immunoabsorption</td>
</tr>
<tr>
<td>Drug resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Phospholipid, Cardiolipin, B2-glycoprotein I</td>
<td>Not reported</td>
</tr>
<tr>
<td>Primary generalised before SLE onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal or generalised-tonic during SLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapy-resistant localisation-related epilepsy</td>
<td>Cardiolipin, nuclear, B2-glycoprotein I, GAD</td>
<td>Not reported</td>
</tr>
<tr>
<td>Newly diagnosed seizure</td>
<td>Cardiolipin, nuclear, B2-glycoprotein I</td>
<td>Not reported</td>
</tr>
<tr>
<td>Generalised epilepsy syndromes</td>
<td>Cardiolipin</td>
<td>Not reported</td>
</tr>
<tr>
<td>West’s syndrome</td>
<td>Haemocyanin</td>
<td>Intravenous therapy</td>
</tr>
<tr>
<td>Cryptogenic Lennox-Gastaut syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completely controlled epilepsy</td>
<td>GAD</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

Modified from Bernasconi et al. 2002

**Antiphospholipid and antinuclear antibodies.** An increased prevalence of aCL and ANA has been reported both in a pediatric and adult epilepsy populations without the antiphospholipid syndrome or SLE. According to Verrot et al. the presence of IgG class aCL was 19% and ANA 25% in a population of 163 adult epilepsy patients (Verrot et al. 1997). In the study of Peltola et al. the presence of IgG class aCL (21%) and ANA (24%) were significantly increased in localisation-related epilepsy in adult patients (Peltola et al. 2000b). Angelini et al. reported 3 aCL positive child epileptics in a series of 23 patients with partial seizures (Angelini et al. 1998). Eriksson et al. reported the presence of aCL to be 44% in a child population with multiple seizure types, mostly symptomatic aetiolo-
gy, early onset and poor seizure control (Eriksson et al. 2001). The presence of ANA was also increased in newly diagnosed patients. Debourdeau et al. have recently reported the presence of aCL and ANA to be the same in a population of 81 patients and control subjects (Debourdeau et al. 2004). In most of the studies patients have come from tertiary centres and control subjects have not come from the general population, but convenient samples have been used instead. An increased presence of anti-B2-GPI-antibodies has been found in some studies as well (Verrot et al. 1997, Peltola et al. 2000b, Eriksson et al. 2001). The prevalences of aCL previously reported in control populations have varied widely and in normal populations have been between 1%–5.6% (Petri 2000).

Epilepsy associated with anti-GAD antibodies. Several investigators have found anti-GAD antibodies in patients with epilepsy, especially in patients with refractory seizures (Solimena et al. 1988, Nemni et al. 1994, Saiz et al. 1997, Giometto et al. 1998, Marcirolo et al. 2001). Peltola et al. found 8 patients out of 51 with anti-GAD antibodies, of which two with high titres were comparable to those seen in SMS (Peltola et al. 2000c). Both had therapy-resistant localisation-related epilepsy. Kwan et al. found no difference between the levels of anti-GAD antibodies in patients with well-controlled and uncontrolled epilepsy (Kwan et al. 2000). It is not known if these antibodies in epilepsy are causative or a secondary phenomenon.

2.3.2 Epilepsy, epilepsy medication and serum immunoglobulin concentrations

Changes in all Ig subtype serum concentrations in patients with epilepsy have been reported in the literature, but a phenytoin-associated decrease of IgA, first reported in the 1970s, is the most consistent finding in various studies (Bardana et al. 1983, Ponti et al. 1993, Aarli 1993, Aarli 2000). The effect of PHT on serum IgA has been shown to develop in 3–4 months after the initiation of therapy and results in a fall of serum IgA concentrations to subnormal levels in some patients (Aarli 1993). This effect has been shown to normalise within 3–4 weeks after cessation of therapy in most patients (Gilhus & Aarli 1981). The effect of PHT on serum IgA is thought to be genetically determined. Its effect may depend on direct action on plasma cells or indirectly through cells which control the production of IgA (Pereira et al. 1983). The clinical significance of lowered serum IgA concentration is debated, but has been associated with increased risk for upper respiratory tract infections and gingival hyperplasia (Aarli 1993). HLA associations in PHT associated IgA deficiency and primary IgA deficiency seen in some patients with epilepsy prior to PHT treatment differ and may implicate different genetic mechanisms. CBZ has been shown to cause a slight fall in the serum concentrations of IgM and IgA (Sorrel & Forbes 1975, Pacifici et al. 1991, Ponti et al. 1993, Basaran et al. 1994). Phenobarbitone, primidone and VPA have also been reported to lead to a slight reduction in serum IgA concentration (Ponti et al. 1993). IgG subclass changes have been reported during phenytoin therapy, as well as a decrease in serum IgE concentration (Aarli 1993). Reports on the effects of Ig serum concentrations other than IgA and associations with drugs other than phenytoin are mostly contradictory. The changes in all Ig serum concentrations in
general have been attributed to the combined and independent effects of pharmacotherapy and genetic susceptibility represented by increased presence of certain HLA phenotypes in reported cases.

### 2.3.3 Epilepsy and coeliac disease

Coeliac disease (CD) is a lifelong intolerance to the protein fraction of wheat (gluten), barley (hordeins) and rye (secalins). Ingestion of these proteins leads to small bowel damage. Severe disease may lead to malabsorption of essential nutrients. Symptomatic patients often develop weight loss and watery diarrhea along with abdominal complaints. Patients can present with iron or folate deficiency, anemia, osteoporosis, osteomalacia, chronic fatigue, intolerance of milk products, dental enamel defects, dementia, short stature, neuropathy, and infertility. CD is one part of a broader spectrum of gluten sensitivity that includes skin (dermatitis herpetiformis), kidney (IgA related nephropathy), and possibly other organ involvements (vide infra) (Elliott et al. 1998).

The prevalence of CD has probably been underestimated, and in recent reports the prevalence has been typically 1:100 and may be higher. It is now widely accepted that diagnosed CD represent only the “tip of the iceberg” of the actual CD in the population (Mäki et al. 1997). Recently a screening approach of relatively specific CD associated antibodies has been used, followed by biopsy to confirm the diagnosis (Farrell & Kelly 2002).

The classical pathological abnormality in the small bowel consists of villous atrophy, crypt hyperplasia and increased numbers of lymphocytes infiltrating the epithelial cell layer and lamina propria. Cytokine-producing T cells are essential for the morphological changes. CD4+ cells mediate the disease. Enzyme tissue transglutaminase is the target of endomysial autoantibody, an essential marker of the disease. This enzyme is expressed on the subepithelial layer of intestinal epithelium, where it deamidates the glutamine residues in gliadin.

The mechanism by which gliadin peptides bind with high affinity T cells has recently become clear. Deamidated peptides adhere strongly to the binding grooves of HLA-DQ2 and DQ8 molecules and elicit strong T cell responses. Proteins responsible for CD carry epitopes that activate T cells. Shan et al. have shown that one 33-amino-acid peptide survives transit through the digestive canal and arrives at the small bowel. This 33-mer has a very high affinity for tTGA and after being deamidated it elicits T-cell response. Similar peptide sequences are present in the hordeins and secalins (McManus & Kelleher 2003).

In addition to the autoantibodies EMA and anti-tTGA, antibodies against gluten are characteristic of CD. These antibodies have also been implicated in the pathogenesis of CD associated neurological disorders through proposed cryptic gliadin sensitivity (Hadjivassiliou et al. 2002). This hypothesis has been criticised, however (Wills 2000, Wills et al. 2002, Wills & Unsworth 2002). In some cases an ameliorating response to a gluten-free diet has been noticed.

Various neurological disorders have been ascribed to CD, including ataxia, sensorimotor axonal neuropathy, mononeuropathy multiplex, motor neuropathy, small fiber neuropathy, mixed demyelinating/axonal neuropathy, myopathies, abnormal white matter, stiff man syndrome and neuromyotonia (Luostarinen et al. 1999, Pellechia et al. 1999, Pen-
giran-Dengah et al. 2002, Jiskra et al. 2003). These reports represent patients from specialised referral centres, however, and do not reflect the real prevalence of these disorders in the population. In addition to gluten sensitivity, several other mechanisms associated with neurological disorders in CD patients have been proposed. These include overt or subclinical malabsorption of folic acid and vitamin B12 and selective vitamin E deficiency (Elliott et al. 1998). Antineuronal antibodies have been reported to be associated with some cases of CD related neurological disorders (Volta et al. 2002).

A high prevalence of epilepsy in CD patients has been suggested, and a specific syndrome of CD, epilepsy and occipital calcifications has been described in Italian populations (Gobbi et al. 1991, Sammaritano et al. 1992, Magaudda et al. 1993). Chapman and co-workers have reported a prevalence of 5% of epilepsy in CD patients (Chapman et al. 1968). The prevalence of CD in patients with epilepsy has been less extensively studied. Cronin et al. reported four CD cases in an epilepsy population of 177 (2.3%) (Cronin et al. 1998). Luostarinen et al. screened 199 patients with epilepsy and found CD cases confirmed with small bowel biopsy (2.5%) (Luostarinen et al. 2001).
3 Aims of the research

The general aim of this study was to elucidate the involvement of autoimmune mechanisms in epilepsy, in particular to assess the occurrence of autoantibodies in patients with epilepsy relative to the rest of the population. The specific aims were

1. to determine the presence of autoantibodies and serum immunoglobulin concentrations in a cohort of representative patients with epilepsy and population based matched controls

2. to identify possible associations between specific autoantibodies, immunoglobulins and epilepsy attributes, co-morbidity and epilepsy medications in patients with epilepsy

3. to determine the presence of CD related antibodies in patients with epilepsy and population based reference subjects and to elucidate possible associations between CD related antibodies and epilepsy attributes, co-morbidity and epilepsy medications in patients with epilepsy

4. to determine possible associations between the immunological parameters studied.
4 Patients and methods

4.1 Study subjects

The study was conducted at the Outpatient Department of Neurology in the Oulu University Hospital with the approval of the Ethics Committee of the Medical Faculty of University of Oulu. The Oulu University Hospital is the primary referral centre for all adult patients with epilepsy from a source adult population of approximately 260,000. The target population consisted of 1386 patients treated for epilepsy in the Oulu University Hospital during the years 1996–7. The patient population represents a comprehensive prevalence sample of adult patients treated for epilepsy in the community. All eligible patients were asked to participate in the study. In all, 968 (70%) patients gave their informed consent and participated in the study. The patient population consisted of 496 male subjects (51.2%) and 472 female subjects (48.8%). The number of patients in each analysis varied slightly depending on the amount of blood sample available for the analyses in each subject.

The demographic data of the patients and reference subjects is presented in Table 7. The type of epilepsy was classified according to the recommendations of the International League Against Epilepsy (ILAE 1989). A great majority of the patients, 86.9%, had partial epilepsy, 9.5% had primary generalised epilepsy and 3.6% unclassified epilepsy. Half of the patients were seizure free (51.0% of patients), 31.4% had less than one seizure per month, 9.4% had 1–3 seizures per month and 8.2% had four or more seizures per month. The most commonly used antiepileptic drugs (mono- and polytherapy combined) were carbamazepine (48.4%), oxcarbazepine (27.5%), valproate (20.1%), lamotrigine (10.4%) and phenytoin (9.0%).

In addition, 1386 control subjects matched for age, gender and municipality of residence were identified from the Population Register Centre and were asked to participate in the study. Of these, 584 (42%) participated. Blood samples were drawn from both patients with epilepsy and control subjects, and their medical records at the Oulu University Hospital were reviewed retrospectively in order to obtain all pertinent medical information.
4.2 Measurements

The laboratory analyses were performed at the Centre for Laboratory Medicine, Tampere University Hospital (aCL, ANA, antimitochondrial antibodies, anti-B-GPI, IgA, IgG, IgM, AGAbA, AGAbG) and at the Pediatric Research Centre, Medical School, University of Tampere, Tampere (tTGAbA, EMA).

4.2.1 aCL and anti-B2-GPI

IgG class aCL and anti-B2-GPI were measured by a commercial enzyme immunoassay (Varelisa Cardiolipin Antibodies and Varelisa B2-glycoprotein I, Pharmacia&Upjohn, Freiburg, Germany). Anti-B2-GPI were analysed from all aCL positive sera. aCL were expressed in GPL units according to the recommendations developed at the 1986 workshop on standardisation of interpretation of anticardiolipin test results (Harris et al. 1994). Values ≥12GPL were considered positive results. Anti-B2-GPI <10U/ml were considered negative, between 10-15U/ml borderline values and >15U/ml positive.

4.2.2 ANA and AMA

IgG ANA were detected by a standard indirect immunofluorescence method using commercial Hep-2 cells as antigens (INOVA Diagnostics, San Diego, California). Sera were diluted 1:80 and 1:160 in PBS and incubated on substrate slides along with the appropriate controls. Fluorescence-conjugated anti-human immunoglobulin antiserum was used as the secondary antibody. The slides were examined using fluorescence microscopy. Any nuclear staining was considered a positive result, and the titres and the staining patterns of the positive sera were determined. Titres ≥80 were considered positi-
Antinuclear antibodies were measured by indirect immunofluorescence using Hep-2 cells. Any with cytoplasmic staining resembling mitochondrial antibodies (AMA) were further tested by a standard indirect immunofluorescence using tissue sections from rat and mouse. The initial serum dilution was 1:50. Antibodies specific for primary biliary cirrhosis (PBC) were studied using a commercial kit.

4.2.3 AGA

IgA and IgG-class antigliadin (AGA) antibodies were measured according to standard enzyme immunoassay with a crude gliadin (Sigma G3375) as antigen (Vainio et al. 1983). The lower limit for positiveness in IgA class was 0.20 EU/ml and in IgG class 10 EU/ml.

4.2.4 tTGAbA

Serum tissue transglutaminase antibodies (tTGAbA) were measured using a commercial kit Quanta Lite tTG ELISA (INOVA Diagnostics, Inc., San Diego, CA, USA) according to the manufacturer's instructions. The antibody concentrations were expressed in arbitrary units (AU), that is, percentages of the positive reference serum, and values >20 AU were considered positive (Mustalahti et al. 2002).

4.2.5 EMA

Serum IgA-class endomysial antibodies (EMA) were determined by an indirect immunofluorescence method using human umbilical cord as antigen, and a serum dilution of 1:5 was considered positive (Sulkanen et al. 1998a, Sulkanen et al. 1998b).

4.2.6 Serum immunoglobulins

Immunoglobulins were measured by a routine nephelometric method using a Behring BN II analyzer. The cut-off values from the manufacturer were used. The normal range for IgA was 0.88-4.84g/L in men and 0.52-4.02g/L in women, for IgG 6.77-15g/L both in men and women and for IgM 0.36-2.59g/L in men and 0.47-2.84g/L in women. Values below these ranges were deemed low, within the limits normal and above the range high. Subjects with IgA serum concentrations below 0.06g/L were deemed IgA deficient.
4.3 Statistical methods

4.3.1 aCL, ANA, anti-B2-GPI and AMA analysis (Studies I and IV)

The odds ratio as the measure of effect was calculated using unconditional logistic regression analysis with likelihood-based confidence intervals. The case was the presence of autoantibodies and the explanatory variables were epilepsy type, age at onset, duration of epilepsy, seizure frequency and antiepileptic medication. Gender, age at observation and co-morbidity were regarded as potential confounders and adjusted by multivariate modeling. Statistical significance was evaluated by two-sided likelihood ratio tests.

4.3.2 Igs and CD-related antibodies (Studies II and III)

A generalised linear model with a binomial error structure and logarithmic link function was used to estimate the prevalence ratio, i.e. relate prevalence of decreased immunoglobulin levels and CD associated antibody levels in patients with epilepsy relative to those in the reference group. Gender, age at observation and co-morbidity were regarded as potential confounders and adjusted by multivariate modeling. Statistical significance was evaluated by two-sided likelihood ratio tests.

4.3.3 Interrelations of different immunological markers (Study IV)

Interrelations of antibodies were analysed by the chi-square test and with Fisher’s two-sided test. Due to a large number of significance tests, a lower significance level was used, corresponding to a Bonferroni correction (a total of 50 significance tests, with adjusted significance level of 0.001).
5 Results

The prevalences of aCL, ANA and AMA in patients with epilepsy and reference subjects are presented in Table 8.

Table 8. Prevalences of aCL, ANA and AMA in patients with epilepsy (n=914) and reference subjects (n=550).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>aCL (&gt;12GPL)</th>
<th>ANA (titre ≥1:80)</th>
<th>AMA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>4.5%</td>
<td>17.3%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Reference subjects</td>
<td>5.0%</td>
<td>16.9%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Statistical significance between</td>
<td>N.S.</td>
<td>N.S.</td>
<td>RR 2.2, CI 1.1-4-1, p=0.03</td>
</tr>
<tr>
<td>patients and reference subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.1 aCL

aCL were present in 4.5% of the patients and 5.0% of the control subjects.

There was no statistically significant difference in the presence of aCL between patients and control subjects. 33 patients with epilepsy and 23 control subjects had positive aCL levels. aCL were twice as common in men as in women, both in the patients and the reference subjects. Long duration and poor seizure control of partial epilepsy were associated with an increase in aCL. Of patients with partial epilepsy for 30 years 8.4% had aCL and these patients were 3.1 times (95% CI 1.2-7.6) more likely to have anticiadiolin antibodies than patients with partial epilepsy for <10 years (prevalence of aCL 2.9%). The prevalence of aCL in patients with epilepsy according to the duration of epilepsy is presented in Figure 1.
Moreover, 7.1% of the patients with partial epilepsy and 1 seizure/month had aCL and these patients were 2.2 times more likely to have aCL than patients with partial epilepsy and <1 seizure/month (prevalence of aCL 3.7%) in logistic regression analysis after adjustment of age, sex and seizure frequency as independent variables (95% CI 1.04-4.7). Only six patients (0.6%) with primary generalised epilepsy had aCL.

5.2 ANA

ANA with predominantly low titres were present in 17.3% of the patients and in 16.9% of the control subjects. Age did not have an effect on the presence of ANA. However, in women ANA positivity was 1.5 times more common than in men both in patients and control subjects. Insulin dependent diabetes was associated with ANA in patients with epilepsy (odds ratio 2.2, 95% CI 1.02-4.7). Patients with 1 seizure/month tended to have an increased presence of ANA (22.3%) compared to patients with <1 seizure/month (15.9%; odds ratio 1.5, 95% CI 0.98-2.2).

The presence of aCL was not associated with ANA.

5.3 anti-B2-GPI

Two patients and one control subject had anti-B2-GPI.
5.4 AMA

Thirty seven patients (3.9%) and 11 control subjects (1.9%) had AMA (RR 2.1, CI 1.05-4.1, p=0.03). In patients these antibodies were associated with longer duration of epilepsy and older age at the onset of epilepsy. Older age at the onset of epilepsy increased the risk for AMA annually by 4.2% (CI 2.0-6.5%, p=0.0002). The annual risk increment of longer duration of epilepsy for AMA was 5% (CI 1.7-8.9%, p=0.004). One epilepsy patient and two control subjects had high positive immunofluorescent titres specific for PBC.

5.5 Serum immunoglobulin concentrations

The prevalence of low, normal and high serum Ig concentrations in patients with epilepsy and reference subjects are presented in Table 9.

Table 9. Serum immunoglobulin (Ig) concentrations in patients with epilepsy (n=958) and reference subjects (n=581)

<table>
<thead>
<tr>
<th>Ig</th>
<th>Serum concentration</th>
<th>Patients</th>
<th>Reference subjects</th>
<th>RR for low Ig Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>Low</td>
<td>79 (8.2%)</td>
<td>11 (1.9%)</td>
<td>4.4 (CI 2.3-8.1)</td>
</tr>
<tr>
<td></td>
<td>Normal*</td>
<td>855 (89.2%)</td>
<td>552 (95.2%)</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>21 (2.2%)</td>
<td>18 (3.1%)</td>
<td>N.A.</td>
</tr>
<tr>
<td>IgG</td>
<td>Low</td>
<td>47 (4.9%)</td>
<td>25 (4.3%)</td>
<td>1.1 (CI 0.7-1.8)</td>
</tr>
<tr>
<td></td>
<td>Normal*</td>
<td>872 (91.0%)</td>
<td>531 (91.4%)</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>39 (4.1%)</td>
<td>25 (4.3%)</td>
<td>N.A.</td>
</tr>
<tr>
<td>IgM</td>
<td>Low</td>
<td>29 (3.0%)</td>
<td>20 (3.4%)</td>
<td>0.9 (CI 0.5-1.5)</td>
</tr>
<tr>
<td></td>
<td>Normal*</td>
<td>899 (93.8%)</td>
<td>554 (95.3%)</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>30 (3.1%)</td>
<td>7 (1.2%)</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

*normal ranges: IgA: 0.88-4.84g/L in men and 0.52-4.02g/L in women
IgG: 6.77-15g/L
IgM: 0.36-2.58g/L in men and 0.47-2.84g/L in women
N.A.= not applicable; RR= risk ratio

5.5.1 IgA

Low serum IgA levels (<0.88mg/l in men and <0.53mg/ml in women) were 4.4 times more common in patients with epilepsy than in control subjects (95% confidence interval (CI) 2.3-8.1 p<0.0001). Low serum IgA concentrations were present in 9.1% of patients with partial epilepsy and in 1.9% of reference subjects (RR 4.7, CI 2.6-8.9, p<0.001). Of patients with primary generalised epilepsy 3.3% had low serum IgA concentrations (RR 1.7, CI 0.50-6.0, p=0.4). The association between partial epilepsy and low serum IgA concentrations is explained by the effect of current or previous phenytoin (PHT) medica-
tion. In a multivariate model combining both partial epilepsy and PHT medication, the significance of partial epilepsy is reduced (p=0.62, OR 1.4, CI 0.3-6.4) while in patients with prior or present PHT the probability for low IgA serum concentrations was increased 2.8 fold (p=0.005, CI 1.4-5.7).

Of patients currently on PHT, 19.1% (RR 10.0, CI 4.8-20.9, p<0.001), had low serum IgA concentrations, compared to a 1.9% prevalence of low serum IgA concentrations among the reference subjects. Among patients taking other major antiepileptic drugs, the range varied from 5.5 to 8.6%. Of patients with previous PHT therapy, 11.9% had low serum IgA, whereas 3.8% of patients who had never been on PHT therapy had low serum IgA. The difference between patients never on PHT and reference subjects is statistically significant (p=0.045, OR 2.2, CI 1.0-4.8). The patients on current PHT medication had a sixfold risk for low IgA when compared to patients with no history of PHT exposure (p<0.001, OR 5.7, CI 2.7-12.2). The effects of current and previous PHT exposure were concordant both in the monotherapy and polytherapy groups.

Three patients (0.3%) and none of the control subjects had IgA deficiency.

### 5.5.2 IgG

No statistically significant differences in serum IgG concentrations were found between the patients and the control subjects.

### 5.5.3 IgM

No statistically significant differences in serum IgM concentrations were found between the patients and the reference subjects.

### 5.5.4 Immunoglobulins, epilepsy attributes and co-morbidity

A multivariate analysis was performed concerning serum Ig concentrations in patients with epilepsy. Phenytoin medication and female gender were the only explanatory variables associated with low serum IgA concentrations. In multivariate analysis low serum IgG concentration (<6.77g/l) was associated with autoimmune disease co-morbidity in patients with epilepsy (RR 3.0, CI 1.6-5.8, p=0.0008). Low serum IgM concentration was associated with higher age at onset of epilepsy (RR 1.04, CI 1.02-1.07, p=0.001), longer duration of epilepsy (RR 1.06, CI 1.02-1.1), valproate medication (RR 3.3, CI 1.3-8.2, p<0.013) and autoimmune diseases (RR 2.7, CI 2.7-6.2, p=0.02). Of patients on valproate therapy, 4.4% had low IgM, whereas 3.4% of control subjects had low IgM (p=0.19, RR 1.3, CI 0.8-4.0) when age and gender were adjusted in the statistical model.
5.6 CD-related antibodies

The prevalence of CD related antibodies in patients with epilepsy and reference subjects is presented in Table 10 and the associated variables in Table 11.

Table 10. The prevalence of coeliac disease related antibodies in patients with epilepsy and the reference subjects.

<table>
<thead>
<tr>
<th>Antibody type</th>
<th>Patients*</th>
<th>Control subjects*</th>
<th>Risk ratio between patients and control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGAbA</td>
<td>121/968</td>
<td>62/584</td>
<td>RR 1.2, CI 0.8-1.9, p=0.45</td>
</tr>
<tr>
<td>AGAbG</td>
<td>53/964</td>
<td>27/584</td>
<td>RR 1.2, CI 0.8-1.9, p=0.45</td>
</tr>
<tr>
<td>tTGAbA</td>
<td>20/853</td>
<td>14/574</td>
<td>RR 0.96, CI 0.5-1.9, p=0.9</td>
</tr>
<tr>
<td>EMA</td>
<td>15/853</td>
<td>13/574</td>
<td>RR 0.8, CI 0.4-1.6, p=0.5</td>
</tr>
</tbody>
</table>

*number of low positive and positive subjects per all samples analysed

Abbreviations: AGAbA Immunoglobulin A class antigliadin antibodies; AGAbG Immunoglobulin G class antigliadin antibodies; tTGAbA Immunoglobulin A class tissue transglutaminase antibodies; EMA endomysium antibodies

Table 11. Independent variables increasing the probability for CD related antibodies in binary logistic regression analysis in patients with epilepsy.

<table>
<thead>
<tr>
<th>Antibody dependent variable</th>
<th>Independent variables in patients with epilepsy</th>
<th>OR 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGAbA</td>
<td>primary generalised epilepsy</td>
<td>2.2</td>
<td>1.2-4.2</td>
</tr>
<tr>
<td></td>
<td>autoimmune thyroid disease</td>
<td>3.3</td>
<td>1.6-6.5</td>
</tr>
<tr>
<td></td>
<td>alcohol abuse</td>
<td>2.2</td>
<td>1.1-4.9</td>
</tr>
<tr>
<td>AGAbG</td>
<td>short duration of epilepsy</td>
<td>1.03</td>
<td>1.00-1.06</td>
</tr>
<tr>
<td></td>
<td>low age at the onset of epilepsy</td>
<td>1.03</td>
<td>1.01-1.05</td>
</tr>
<tr>
<td>EMA</td>
<td>no seizures</td>
<td>6.3</td>
<td>1.4-25</td>
</tr>
<tr>
<td></td>
<td>autoimmune disorders</td>
<td>4.0</td>
<td>1.3-12.2</td>
</tr>
</tbody>
</table>

Abbreviations: AGAbA Immunoglobulin A class antigliadin antibodies; AGAbG Immunoglobulin G class antigliadin antibodies; tTGAbA Immunoglobulin A class tissue transglutaminase antibodies; EMA endomysium antibodies

5.6.1 AGAbA

AGAbA were analysed in 968 patients and 584 control subjects. One hundred twenty-one patients (12.5%) and 62 control subjects (10.6%) had positive or low positive AGAbA (p=0.256). In the patient population the presence of AGAbA antibodies was associated with primary generalised epilepsy. Patients with partial epilepsy were 0.45 times less likely to have AGAbA than patients with primary generalised epilepsy (p=0.015, 95% CI 0.24-0.85). Moreover, patients with autoimmune thyroid disease were 3.3 times more likely to have AGAbA (p=0.001, 95% CI 1.6-6.5) and alcohol abuse increased the probability 2.2 times (p=0.033, 95% CI 1.1-4.9). The probability to have AGAbA was decreased by 0.615 (p=0.025, 95% CI 0.4-0.9) in patients taking CBZ, which is probably explained by the preference of CBZ in partial epilepsy. Age increased the probability of AGAbA by 1.7% (p=0.015, 95% CI 1.00-1.03).
5.6.2 AGAbG

AGAbG were analysed in 964 patients and 584 control subjects. Fifty-three (5.5%) of the patients and 27 (4.6%) of the control subjects had AGAbG (p=0.45). Patients with longer duration of epilepsy were 0.97 times less likely to have AGAbG than patients with a shorter duration (p=0.025, 95% CI 0.94-0.996) and patients with an older age at the onset of epilepsy were also 0.97 times less likely to have AGAbG (p=0.002, 95% CI 0.95-0.99). No association was found between epilepsy type and the presence of AGAbG.

5.6.3 Tissue transglutaminase antibodies

tTGAbA were analysed in 853 patients and 574 control subjects. Twenty patients (2.3%) and 14 control subjects (2.4%) had low positive or positive tTGAbA (RR 0.96, CI 0.5-1.9, p=0.9)).

5.6.4 Endomysial antibodies

EMA were analysed in 853 patients and 574 control subjects. Fifteen patients (1.8%) and 13 control subjects (2.3%) had EMA (p=0.5). Patients with seizures during the preceding year were 0.16 times less likely to have EMA (p=0.017, 95% CI 0.04-0.72) than patients without seizures. Patients with autoimmune disorders other than thyroid disorders were 4.0 times more likely to have EMA (p=0.014, CI 1.3-12.2). All 15 patients with EMA had partial epilepsy.

5.7 Co-presence of the immunological markers in patients with epilepsy and in reference subjects

There were only a few associations between the immunological markers studied. Low Ig serum concentrations were interrelated among the patients with epilepsy (low IgA and IgG, p<0.001, low IgA and IgM, p=0.007, as well as low IgG and IgM, p<0.001). In the reference population only low IgG and IgM were associated (p=0.008), but the number of subjects with other Ig subgroups was low. As expected, various CD-related antibodies were associated with each other in both the patients and the reference subjects. In the reference population, all CD-related antibodies were associated with aCL (AGAbA and aCL, p=0.002, AGAbG and aCL, p=0.008, aCL and rTGAbA, p=0.003, aCL and EMA, p=0.022), but not in the patient population.
6 Discussion

6.1 General aspects

Epilepsy is a common disorder and is associated with significant co-morbidity with other diseases including disorders with proven or suspected autoimmune origin (Aarli 2000). The presence of various autoimmune disease associated antibodies has previously been reported in epilepsy patients. In most studies, however, patients have been recruited from tertiary centres and reference populations have not been population-based. The results of the present study revealed no major differences between patients with epilepsy and reference subjects in the immunological markers studied, with the exception of unspecific AMA (IV) and low IgA serum concentrations associated with previous and current phenytoin medication (II). This finding in itself suggests that epilepsy related autoimmune phenomena are restricted to selected patient groups. This may most probably have also been the case in the previous, smaller studies. The present results indicate that frequent seizures and long duration of epilepsy may induce changes in the immune system as seen with the association of aCL with long duration of epilepsy and poor seizure control (I). Although the patient population and the reference population differed in size, the reference population was sizeable (n=584). The gender difference between study populations was adjusted for in the multivariate analyses. Therefore risk for bias in the results should be small.

aCL and anti-\( \beta \)-2-GPI have been associated with vascular events in SLE patients and in patients with both epilepsy and SLE (Herranz et al. 1994, Toubi et al. 1995, Liou et al. 1996). ANA at high titres are a hallmark in the diagnosis of connective tissue diseases. As a whole, the antibody concentrations determined in the present study were low, and the occurrence of anti-\( \beta \)-2-GPI was not substantially elevated (I). This implies that antibody formation is a nonspecific immunological by-product of seizures and that these antibodies are not of clinical significance, but represent natural autoantibodies (Alarcon-Segovia & Cabral 1998). Associations between the antibodies studied and autoimmune disorders were scarce in the present study being most evident with CD-related antibodies (III). In addition, interrelations between the immunological parameters studied were few (IV).
Low IgA serum concentrations were more common in patients with epilepsy (II). This result is concordant to previous studies (Aarli 1993, 2000). However, association of low serum IgA levels with previous PHT medication implies phenytoin exerts permanent immunological changes in a proportion of patients exposed to it. PHT was the only major antiepileptic drug associated with low serum IgA concentrations.

CD is a common autoimmune disease and the present results suggest it to be as common in patients with epilepsy as in the population-based reference group with the prevalence of probable CD approaching two percent based on EMA and tTGA prevalence (III). In general, the associations between CD related antibodies and autoimmune disorders in our study are well in concordance with previously reported findings (Lang et al. 2003). Antigliadin antibodies are relatively unspecific for CD and may be associated with other immunological phenomena. AGAbA were associated with primary generalised epilepsy, which may indicate a genetic susceptibility to antibody formation.

As expected, low Igs were interrelated in patients as were CD-related antibodies. Otherwise the immunological markers studied were not interrelated. The associations found in the present study seem to reflect some interaction of genetic susceptibility and epilepsy per se, especially frequent seizures, on immunological manifestations and selected parameters in patients with epilepsy.

6.2 Frequencies of aCL, ANA and anti-B2-GPI

The main finding was the similar occurrence of aCL and ANA in patients with epilepsy and in the reference subjects. The prevalence of aCL observed both in patients with epilepsy and in control subjects is in agreement with the reported frequencies in various populations (Peltola et al. 2000b), where the reported variation has ranged from 1.0 to 5.6%. Anti-B2-GPI were not present in a significant number of study subjects. aCL levels were low as were ANA titres. This suggests that antibodies in the patient and reference populations represent natural autoantibodies (Alarcon-Segovia & Cabral, 1998), and are unlikely to be of clinical significance, which is further supported by the small number of anti-B2-GPI antibody positive study subjects. The similar presence of autoantibodies in epilepsy patients and reference subjects and the low concentrations of antibodies observed indicate that autoimmune dysregulation is not a common feature in epilepsy. By contrast, previous studies have reported increased frequencies of aCL and ANA in pediatric and adult populations (Verrot et al. 1997; Angelini et al. 1998, Peltola et al. 2000b, Yoshimura et al. 2001, Eriksson et al. 2001). However, patients in these studies have mainly been recruited from tertiary centres and reference groups have not been population based, which could explain the discrepancy between the present findings and previous reports.
6.2.1 Epilepsy attributes and aCL, ANA and anti-B2-GPI

A high prevalence of aCL was associated with long duration of epilepsy, most explicitly when epilepsy had lasted over 30 years, and also with suboptimal seizure control. In addition, suboptimal seizure control tended to increase the presence of ANA. This may reflect the effect of seizures on the autoimmune system. Recent seizures have been reported to be associated with autoantibodies (Peltola et al. 2000b), and seizures are known to activate cytokine production (Peltola et al. 2000a), which could contribute to increased autoantibody production.

In the present study most patients had well-controlled partial epilepsy. However, no associations between epilepsy type in general and the presence of autoantibodies were found, in contrast to what has been reported previously (Verrot et al. 1997). One previous report has suggested an increased prevalence of aCL and ANA in adult patients with localisation related epilepsy (Peltola et al. 2000b), but the reported high frequencies of autoantibodies in patients with epilepsy in the previous studies may reflect the high number of patients with poor seizure control participating in those studies. It is generally recognised that patients with poor seizure control have partial epilepsy in most cases.

6.2.2 Antiepileptic medication, aCL, ANA and anti-B2-GPI

Antiepileptic medication did not have an effect on the presence of aCL and ANA, which is in accordance with other recent reports (Verrot et al. 1997, Peltola et al. 2000b, Eriksson et al. 2001). The only association found was that between clonazepam monotherapy and aCL in a negligibly small number of patients. Thus, commonly used antiepileptic drugs do not seem to have a significant role in inducing antibody production in patients with epilepsy.

6.2.3 Co-morbidity and aCL, ANA and anti-B2-GPI

Associations between ANA, aCL and autoimmune mediated disorders in general were not found, although antinuclear antibodies were associated with insulin dependent diabetes in patients with epilepsy. The prevalence of IDDM did not differ significantly between patients and control subjects, however.

6.3 AMA

AMA were more frequent in the patient population than in controls. In patients these antibodies were associated with long duration of epilepsy and old age at the onset of epilepsy. AMA found in the patient population were not specific for primary biliary cirrhosis.
(PBC) and are most likely a sign of immunological activation related to long epilepsy
duration analogous to the association found between aCL and long duration of epilepsy.
AMA were not associated with co-morbidity or antiepileptic medications. (IV)
AMA have previously been associated with various drugs (Homberg et al. 1985,
Ohmoto et al. 1999) and bacterial infections (Butler et al. 1995) as well as with myocar-
dial infarction (Schifter et al. 1996) and chronic graft-versus host disease (Siegert et al.
1991). The association between liver diseases, especially PBC, and specific AMA has
been well established (Klatskin & Kantor 1972, Mackay 2000). However, only one
AMA-positive patient and two reference subjects in the present study had PBC-specific
AMA. A weak correlation was found between the duration of epilepsy and the age at the
onset of epilepsy and AMA, but there were no associations of AMA with co-morbidity or
antiepileptic drugs. This may implicate the role of seizures in inducing non-specific AMA
formation. The lack of associations between AMA and the other immunological para-
eters studied suggests that these AMA are not clinically significant.

6.4 Serum immunoglobulin concentrations

6.4.1 IgA

Low serum IgA concentrations were more common in patients with epilepsy than in the
reference population. This finding was mainly due to previous and current PHT exposure
in the patient population. On the other hand, partial epilepsy was also associated with low
serum IgA concentrations as was use of any major antiepileptic drug. However, the associ-
ation between partial epilepsy and low IgA could be attributed to previous and current
PHT medication. Current PHT treatment was associated with the highest prevalence of
low serum IgA levels. In addition, PHT was the only drug associated with low serum IgA
concentrations in multivariate analysis comprising different medications in patients with
epilepsy.

Low serum IgA concentrations have been reported to be associated with autoimmune
disorders (Aarli 1993), but no associations with co-morbidity were found in the present
study in patients with epilepsy. Three patients had an IgA deficiency, while in the refe-
rence population there were no subjects with IgA deficiency. These results are in accord-
ance with the majority of previously published studies (Aarli 1993, Bardana et al. 1983,
Ponti et al. 1993).

PHT has previously been shown to be associated with decreased serum IgA concentra-
tions that are reversible after the medication is discontinued (Gilhus & Aarli 1981, Bar-
dana et al. 1983), and it may have an effect on the maturation of B-lymphocytes (Pereira
et al. 1983). This propensity may explain the pronounced effect of PHT on IgA concentra-
tions shown in this study, which may well be unique to this molecule. Our finding of the
association between previous PHT exposure and current low serum IgA concentrations
contradict previous reports on reversible PHT associated IgA changes (Gilhus & Aarli
1981, Bardana et al. 1983). The strong PHT effect on IgA may mask some more subtle
effects of e.g. epilepsy type and frequently occurring seizures that also could affect Ig concentrations.

6.4.2 IgG and IgM

Serum IgG and IgM concentrations were similar in patients with epilepsy and the reference subjects. Previously published findings have been conflicting, reporting increased, decreased or unchanged serum concentrations of these immunoglobulins in patients with epilepsy (Aarli 1993, Bardana et al. 1983, Ponti et al. 1993). However, low serum IgG concentrations were associated with autoimmune diseases and low IgM serum concentrations with higher age at the onset of epilepsy, longer duration of epilepsy and autoimmune diseases in patients with epilepsy. Association between autoimmune disorders and low IgG and IgM serum concentrations may reflect a common genetic susceptibility. Epilepsy attributes affecting IgM serum concentrations may implicate seizures in inducing changes in the immunological system possibly analogous to those seen in increased prevalence of aCL with long duration of epilepsy.

6.5 CD related antibodies

The presence of CD related antibodies was similar in patients with epilepsy and the reference population. This finding suggests that the prevalence of CD is most probably similar in the two study populations and epilepsy and CD are not associated. This finding contradicts some, but not all previous studies (Vascotto et al. 1997, Holmes 1997). EMA and tTGAbA are specific and sensitive markers of CD and the similar prevalence of these antibodies in epilepsy patients and the reference population may predict that the number of undiagnosed cases of CD is similar in these two populations. The presence of both specific autoantibodies was approximately two percent in both study populations, which indicates that the prevalence of undiagnosed CD is likely to approach this figure in the general population. Previously reported prevalences of CD in various populations have been approximately one percent (Johnston et al. 1997, Kolho et al. 1998, Meloni et al. 1999, Rutz et al. 2002, Fasano et al. 2003). According to recent views, the majority of patients suffering from CD are not diagnosed (Mäki 1997). Both epilepsy and CD are common in the general population and the probability for random co-morbidity is therefore considerable.

Antibodies against gluten are less specific diagnostic markers of CD than EMA and TGA. AGA were associated both with epilepsy attributes and autoimmune disorders. In patients with primary generalised epilepsy AGAbA were more frequent than in the reference population. In multivariate analysis of patients with epilepsy, AGAbA were associated with autoimmune thyroid disease, short duration of epilepsy and lower age at the onset epilepsy. Antibodies against gliadin are more abundant and less specific disease markers and therefore the association with other autoimmune disorders is more evident than with the more CD-specific EMA and tTGA. The association between AGAbA and
primary generalised epilepsy as well as AGAbG and short duration and younger age of onset of epilepsy (which both reflect a possible primary generalised background) implicate a possible common genetic susceptibility to primary generalised seizures and antibody formation. The prevalence of antigliadin antibodies was similar in the two study groups, which does not support the suggested theory of cryptic gliadin sensitivity and a pathogenic role of these antibodies in various neurological disorders. EMA were associated with seizure-freedom and autoimmune disorders. However, the number of EMA positive study subjects was low, and strong conclusions cannot be drawn. The association between seizure freedom and EMA is not in concordance with the effect of seizures on other antibodies observed in this study.

The predicted comparable prevalences of CD in the two sizeable study populations contradicts the previous reports on increased prevalence of CD in patients with epilepsy. The different findings in the studies may be explained by differences in patient populations: in the present study the patient population was large and unselected, whereas the previous studies may have included smaller, selected populations of patients with epilepsy.

6.6 Interrelations of the immunological markers studied

There were only a few associations between the immunological parameters studied. To our knowledge associations between these parameters have not previously been studied in patients with epilepsy. Low Igs were interrelated, and this would most probably have been the case also in the reference population if the number of reference subjects had been higher. As expected, CD-related antibodies were associated with each other. aCL and CD-related antibodies were associated in the reference population, but not in patients with epilepsy. This observation is surprising and not readily explained by any previous findings. Few associations found between the large number of disease markers studied exclude common, epilepsy associated or genetic mechanisms in the regulation of antibody formation in patients with epilepsy. Immunological regulation of each marker seems to occur independently, based on different combinations of genetic susceptibility and environmental as well as epilepsy related factors. (IV)
7 Conclusions

Autoimmune mechanisms are not commonly involved in epilepsy and the occurrence of autoantibodies in patients with epilepsy does not differ from that seen in the general population. More specifically,

1. The prevalence of aCL and ANA is similar in patients with epilepsy and control subjects, but AMA are slightly more common in patients with epilepsy. Low IgA is more common in patients with epilepsy than in the reference subjects, but there is no difference in the IgG and IgM serum concentrations.

2. aCL appear to be associated with long duration of epilepsy and poor seizure control in patients with partial epilepsy. This may be related to the immunological effects of seizures. AMA are also associated with long duration of epilepsy and, in addition, with higher age at the onset of epilepsy, suggesting an effect of seizures on immunological regulation as well. Low serum concentrations of IgA are associated with previous and ongoing PHT exposure suggesting long-standing immunological changes induced by PHT in some patients.

3. The prevalence of CD related antibodies does not differ between patients with epilepsy and reference subjects suggesting the prevalence of CD to be the same in the study populations. AGAbA, the least specific disease marker for CD and more readily influenced by various immunological phenomena, are associated with primary generalised epilepsy.

4. In general, the immunological parameters studied do not seem to be interrelated suggesting that independent mechanisms control their presence in the study populations.
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WR, Fischer JH, Bourgeois B, Wilner A, Faught RE, Sachdeo RC, Beydoun A & Glauser TA 
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